TOBACCO INDUSTRY RESEARCH COMMITTEE

#267

APPLICATION FOR SUPPORT OF RESEARCH PROJECT

Date: February 19, 1960

1. Name of Investigator: Christopher M. Martin, M. D.

2. Title: Assistant Professor of Medicine, and Director, Division of Infectious Diseases

3. Institution and Address:
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4. Project or Subject: INTERACTIONS OF VIRUSES AND SUBSTANCES IN TOBACCO SMOKE CONDENSATE

5. Detailed Plan of Procedure:

It is proposed to study, in vitro and in vivo, the interactions of common human respiratory tract viruses and substances present in tobacco smoke. It is planned to study specifically (1) the degree to which whole virus or infectious viral nucleic acid will bind substances in crude condensates of tobacco smoke; (2) the effects in susceptible small animals and in tissue cultures of, respectively, virus alone, virus and concurrently administered smoke condensate, smoke condensate alone, and virus pre-treated with smoke condensate.

If clear-cut evidence of virus-smoke condensate interactions is obtained, or if various combinations of virus and smoke condensate induce unusual cellular necrotizing, inflammatory, or proliferative effects suggestive of synergism, then one or more of the following lines of investigation will be followed: (1) Fractionation of the condensate to determine the nature of the interacting substance; (2) attempts to duplicate interactions using already identified components of smoke condensate (trace metals, nicotine, polycyclic hydrocarbons, aldehydes, etc.); (3) Attempts to block synergistic effects (in animals) by prior immunization of the animal against the virus involved.

(A) Binding of Smoke Condensate substances by Viruses in vitro:
High titer suspensions of the following respiratory and oropharyngeal viruses infectious for man will be prepared: Influenza A and B; Hemadsorption Viruses Types I and II; Herpes simplex; Coxsackie A_9 and B_1; Adenoviruses Types 1, 3, and 7; JH virus; Coe virus. Each will be equilibrated at 37° C. and at refrigerator temperature with solutions of crude smoke condensate, and the mixtures sedimented in a Spinco Model L Ultracentrifuge. The resulting sediments and supernatants will be examined and compared with those of unequilibrated control suspensions by paper chromatographic methods, spectrophotometry, spectrofluorometry, fluorescence microscopy, and trace metal analysis of ashed aliquots.

Similar binding experiments using infectious viral nucleic acid of low protein content will be performed.

(B) Interactions in vivo:
The effects of virus alone, of virus and concurrently administered smoke condensate, of smoke condensate alone, and of virus pre-equilibrated with smoke condensate will be examined in the following systems:

Virus	Animal, Route of Infection	Tissue Culture
Influenza A	Mouse, intranasal	Monkey kidney, Chick chorioallantois, Mouse lung. Human embryonic tracheal epithelium, lung, kidney
Hemadsorption Virus I & II	---	Human embryonic kidney, Monkey kidney
Herpes Simplex	Mouse, intranasal, Hamster, intraoral	Human embryonic kidney, trachea, lung
Coxsackie A_9	---	Human embryonic kidney
Coxsackie B_1	Young adult mice, intranasal, intraperitoneal	" " "
Adenovirus	---	Human embryonic kidney

6. Proposed Budget:

	1st Year	2nd Year
Salaries	$3,000	3,000
Expendable supplies	2,000	2,000
Permanent Equipment	5,000	2,000
Overhead	750	750
Other	--	--
Total	$10,750	7,750

7. Anticipated duration of work:
2 years: April 1, 1960 - March 31, 1962

8. Facilities and Staff available:
Facilities: Remodeling of the permanent laboratories of the Division of Infectious Diseases, located in the Center Building of the Jersey City Medical Center, is proceeding well ahead of schedule. Nine rooms, encompassing 2100 square feet of floor space, have been assigned to the Division. Remodeling of 6 rooms (1600 sq.ft.) in the form of two offices, three laboratories, and a glassware preparation room, will be completed April 1, 1960. Spacious animal quarters are available on top floor, same building. Equipment includes freezer, refrigerator, incubator, storage, and other tissue culture facilities; extensive fume hood space; centrifuges, spectrophotometer, automatic precision balance. Radioisotope equipment, chromatography equipment, and a muffle furnace will be purchased with funds awarded in support of USPHS Research Grant E-3257 -- see below.
Personnel: Investigator, 2 technicians, secretary, glassware diener, and, starting July 1, 1 research fellow (one year) and two medical students (3 months each).

9. Additional Requirements (Explanation of Budget Plan):
(a) Salaries ($6,000.-) - major support of one technician for two years.
(b) Permanent equipment ($7,000.-) portion of cost of Spinco Model L Ultracentrifuge ($3,000.-) and of Aminco Spectrophotofluorometer ($3,000.-).

10. Additional information (including relation of work to other projects and other sources of supply):

(A) Relation of Work to other projects:

The project proposed in this application represents a specific application of several broad concepts to be studied along fundamental lines under USPHS Research Grant E-3257, "Transferrin-Virus-Metal-Carcinogen-DNA Interactions", for which the Division of Infectious Diseases has been awarded $18,724 for the first year of study.*

Topics being studied under E-3257 include:

(a) Nature of the transferrin-sensitive reaction in viral synthesis.
(b) Trace metals in virus replication and nucleic acid formation.
(c) Physiologic intracellular function of transferrin.
(d) Properties, mode of action of transferrin-like antiviral compounds.
(e) Carcinogen-metal complexes and their affinity for nucleic acids.
(f) Carcinogen-binding by viruses.

(B) Background of proposed study:

Data developed in the past two years at Harvard Medical School and reported to the American Society for Clinical Investigation in May, 1959 indicate that transferrin (iron-binding protein) profoundly depresses the synthesis of viral nucleic acid by infected cells through intracellular binding of trace metals other than iron (copper and manganese)[1]. Subsequent studies have demonstrated the intimate association of trace metals with viral nucleic acid chains; preliminary binding studies suggest that in the presence of trace metals viruses become extremely reactive chemicals, binding avidly to nucleic acids and to polycyclic hydrocarbons.

These observations appeared relevant to certain disparate reports by others; Rous and Friedewald in 1944[2] and Duran-Reynals in 1952[3] described erratic synergistic effects between viruses and organic carcinogens. Many investigators, in attempting to produce lung tumors or chronic bronchitis experimentally, using tobacco smoke condensates, e.g. Leuchtenberger, C., et.al.(1958),[4] have noted that most effects occur only after massive exposure to the condensates, and that, in any event, metaplastic or other changes occur in a relatively non-reproducible way, not clearly related to intensity or duration of exposure. Again, in commenting on the carcinogenic properties of certain polycyclic hydrocarbons identified in tobacco smoke condensate in extremely low concentration, Wynder and Hoffman[5] conclude that "initiating" or "promoting" substances must be invoked or assumed to assign these hydrocarbons a major etiologic role in tumorigenesis.

*For background and details of previous studies conducted at the Thorndike Memorial Laboratory, Harvard Medical School, kindly refer to our earlier grant request submitted to the Committee, dated October 6, 1959, and to the copy of USPHS Research Grant Application E-3257 appended to that request.

(C) Significance of Proposed Research:

The present study, exploring the specific relationship between tobacco smoke, common respiratory viruses, and inflammatory and proliferative disease of the lung is designed to examine the possibility that viruses may serve as vectors for delivering toxic substances in tobacco smoke to intracellular loci, thereby initiating or triggering chronic disease.

If such interactions can be satisfactorily demonstrated, they would carry the implication that chronic inflammatory or proliferative disease of the lung is preventable (through virus vaccines) and that minute quantities of toxic substances in tobacco smoke are relatively tolerable.

1. Martin, C. M., and Jandl, J. H., Inhibition of virus multiplication by transferrin, J. Clin. Invest., 38: 1024, 1959.
2. Rous, P., and Friedewald, W. F. The effect of chemical carcinogens on virus-induced rabbit papillomas, J. Exp. Med. 79: 511, 1944.
3. Duran-Reynals, F. Studies on the combined effects of fowl pox virus and methylcholanthrene in chickens, Ann. N. Y. Acad. Sci., 54: 977, 1952
4. Leuchtenberger, C. et al. Correlated histological, cytological, and cytochemical study of tracheobronchial tree and lungs of mice exposed to cigarette smoke: Bronchitis with atypical epithelial changes in mice exposed to cigarette smoke, Cancer 11: 490, 1958.
5. Wynder, E. L., and Hoffmann, D. A study of tobacco carcinogenesis: VII. The role of higher polycyclic hydrocarbons. Cancer, 12: 1079, 1959.

Signature Christopher M. Martin, MD
Director of Project

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